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RUMINANT BACTERIA SEQUESTER COD FROM CHICKEN-PROCESSING WASTEWATER

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This paper reports the effectiveness of un-acclimatized bovine rectal bacteria (BRB) in eliminating COD from chicken-processing wastewater. The results of shake flask experiments using 1d digestion period suggest the ruminant microbes have the potential to sequester 21% COD for an initial COD/SO_4 ratio of 7.8 using the mechanism affiliated to aerobic heterotrophic oxidation. Some 60% removal of lignin/tannin was observed although the microbes appeared adamant not to remove colour. Based on the substrate consumption of 250mg COD per litre per day, the specific microbial growth rate was estimated to be 0.40 per day; specific substrate utilization rate 1.2g COD per g cells per day; net yield 0.42g cells per g COD; and specific cells growth less than 50% per day.

Keywords: Bovine rectal bacteria; ORP; sulphate reduction; sulphide oxidation; chicken-processing wastewater

1. INTRODUCTION

Bacterial contamination in beef has been a subject of much research due to persistent E. coli harbouring the rectal mucosa [1]. To our knowledge however, no work has so far been reported in the literature that has employed bovine rectum bacteria for application in anaerobic wastewater treatment or effluent remediation. This present work attempts to examine the principal mechanisms by which carbon may be removed from chicken-processing wastewater. This wastewater and its associate, the restaurant kitchen-sink wastewater, are two of the polluting activities that most concern our Division of Environment (DoE) Malaysia for the reason that these wastewaters have been rampantly and uncontrollably discharged to the surface drains [2] which subsequently carry the polluting loads to the receiving waters. The chicken-processing operators are never an easy target for the pollution abatement officers because they seclude regulatory surveillance by working in camouflaged, make-shift, and catch-if-you-can type of slaughterhouses.

Table 1: Half reactions for acetate, O_2/SO_4 and cells synthesis

$1/8\ CH_3COO^- + 3/8\ H_2O = 1/8\ CO_2 + 1/8\ HCO_3^- + H^+ + e^-$	(1)
$¼\ O_2 + H^+ + e^- = ½\ H_2O$	(2)
$1/8\ SO_4^{2-} + 19/16\ H^+ + e^- = 1/16\ H_2S + 1/16\ HS^- + ½\ H_2O$	(3)
$1/5\ CO_2 + 1/20\ HCO_3^- + 1/20\ NH_4^+ + H^+ + e- = 1/20\ C_5H_7NO_2 + 9/20\ H_2O$	(4)

To describe the complex nature of the bacterially mediated processes in such applications, it is best to illustrate using half reactions for acetate (Table 1). Equation 1 shows acetate-^{1} oxidation to CO_2. Equations (2) and (3) show the reduction of O_2 and SO_4 respectively to water and sulphide. Equation 4 shows the incorporation of inorganic carbon and nitrogen in the cell material, $C_5H_7NO_2$ [3]. These reactions describe how acetate^{1-} is solubilised by the microbial activities which transfer the energy (reducing electrons) from acetate (electron donor) to oxygen or sulphate (as electron acceptor). Equation (5) shows how acetate is aerobically consumed, producing new cells and carbon dioxide, based on the assumption 59% of acetate is used for synthesis and the remaining 41% for energy production [3].

$$0.125\ CH_3COO^- + 0.1025\ O_2 + 0.0295\ NH4^+ \rightarrow 0.0295\ C_5H_7O_2N + 0.007\ CO_2 + 0.0955\ HCO_3^- + 0.0955\ H_2O \quad (5)$$

There are many electron donors in nature but only few acceptors. Examples of organic donors in heterotrophic metabolism are domestic wastewater protein ($C_{10}H_{19}O_3N$) and carbohydrate (CH_2O) and industrial chemicals such as acetate (CH_3COO^-) and methanol (CH_3OH). Examples of inorganic donors in autotrophic

metabolism are Fe^{2+}, NH_4^+, H_2S, and H_2 [3], [4]. In bacterially mediated processes in wastewater treatment, the bacteria, serving as bio-catalysts, require O_2, NO_3^-, SO_4^{2-}, Fe^{3+} and CO_2 (as electron acceptors) in this preferential order. Energy flowing from a donor to an acceptor may be indicated by measuring oxidation-reduction potential (ORP) as voltage [4]. Positive mV infers energy is towards O_2 and negative mV infers energy is towards NO_3^-, SO_4^{2-}, Fe^{3+} or CO_2. Anaerobic reactions are slower (less efficient) compared to aerobic reactions but may be accelerated using donors with large –mV (for example, cysteine with -480mV and titanium (III) citrate with -480mV) as applied in fermentation processes [5], converting the insoluble organics to soluble organics, volatile acids, carbon dioxide and H_2 and eventually to $CH_4 + CO_2$ and more bacterial cells. The major intermediates formed, acetate and H_2, become highly competitive electron donors between the methane bacteria (during methanogenesis) and the sulphate-reducing bacteria (SRB) (during sulfidogenesis) in reducing sulphate in concentrations as low as 60-150mM [6]. Such competition depends on the COD to SO_4^{2-} mass ratios [7]: ratio 1.5 favours sulfidogenesis and ratio 6.0 favours methanogenesis [6]. The effect of acetate/citrate was investigated using SRB of contaminated aquifer origin [8]. In another study [9] using dried algal biomass as carbon source, COD removal was found to increase with increasing COD to SO_4^{2-} ratio, effecting 31% COD removal via sulphate reduction. Yet other researchers [10] focussed on microbial competition for acetate carbon in an UASB reactor and found at pH < 7.5 the microbes, the sulphate reducers (SRB) and sulphide-oxidizing bacteria (SOB), were affected by H_2S concentrations; and at higher pH, the SRB out-competed the methane bacteria. Another study [11] shows the SRB out-competed the methanogens for CH_3COO^- and H_2 donors when SO_4^{2-} was present in sufficient quantities; the reverse however occurred when SO_4^{2-} was limited and reduction of SO_4^{2-} was inhibited when H_2S was less than 450mg/L (as S).

2. METHODOLOGY

A consortium of bovine rectal bacteria (BRB), obtained from a healthy cow at a local feedlot, was mixed with de-ionized water (DW) to form bacterial slurry. After settlement (3d) at 4^0C, the slurry supernatant was filtered and the solids captured were analysed for total suspended solids (TSS) and volatile suspended solids (VSS). The bacterial cells of known weight were mixed with chicken-processing wastewater in a shake flask which was shaken for 24h at room temperatures ((20 +/- 20^0C). The mixed liquor (before agitation) and its supernatant (after digestion) were analysed for soluble COD, soluble SO_4^{2-}, soluble S^{2-} (plus pH, DO, alkalinity, and temperature) following the HACH DR-5000 Spectrophotometer procedures [12] respectively: Method 8000 (Digestion Method); Method 8051; and Method 8131 (Methylene Blue Method). Alkalinity tests were also performed by titration to pH 4.3 using 0.02N H_2SO_4 titrant [12]. Soluble NO_3-N, NO_2-N, lignin/tannin, and colour (Pt-Co) of the mixed liquor (before digestion) and the mixed liquor supernatant (after digestion) were measured using the HACH DR5000 test procedures.

3. RESULTS AND DISCUSSION

The mixed liquor chicken-processing wastewater COD was initially 833mg/L but decreased to 483mg/L, indicating 42% removal for an initial COD/SO_4 ratio of 7.8. Lignin/tannin decreased from 15mg/L to 6mg/L, inferring 60% removal. Sulphate increased marginally from 240 to 256mg/L with negligible sulphide oxidation. It is deduced COD removal occurred via aerobic heterotrophic oxidation made possible by ample DO (7.5mg/L). The pH started low at 3.7mg/L but increased to 6.4. The microbes sequestered lignin/tannin (60%) but seemed adamant not to remove colour.

The following provides calculation illustrating some of the microbial parameters obtained thus far. Mass of the viable cells (VSS) in the mixed liquor before reaction was 12.7mg, equivalent to (using 20mL samples): X1 = (12.7/20)(1000) = 635mg/L. Mass of the cells after reaction was 21.7mg, equivalent to: X2 = (21.7/20)(1000)= 1088 mg/L. COD removed per day, ΔS = 1872 less 852=1020mg/L. Cells formed (synthesised), $\Delta X = X2 - X1 = 1085 - 635 = 450$mg/L. Cells yield, Y may be calculated from cells growth rate, $dX/dt = Y(-dS/dt) - bX$ where X=635mg/L; dt=1d; and b (decay) is assumed negligible. Thus $Y = dX/dS = 450/1020 = 0.44$mg cells synthesised per day/mg COD (food) utilized=0.44 per day. Specific cells growth = $(\Delta X/\Delta t)/X1 = 450/635 = 0.71$ per day, inferring the cells multiplied by about 70% in one day. Specific substrate utilization rate, $(\Delta S/\Delta t)/X1 = 1020/653 = 1.56$g COD utilised/g cells. In other words, the cells consumed food about 1.6 times their own weight per day

4. CONCLUSIONS

The experimental results show ruminant bacteria could be used to remove COD from chicken-processing wastewater via aerobic heterotrophic oxidation. In contrast to our previous work, COD removal for acetate and citrate relied on sulphate reduction and sulphide oxidation symbiosis. The detention time of 1d proved inadequate for the bacteria to remove colour from the chicken-processing wastewater. The microbes display dependency upon appropriate COD/SO_4 ratio to instigate COD removal.

5. ACKNOWLEDGEMENTS

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[12] HACH DR5000 Spectrophotometer Standard Operation Procedure